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UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.

99,305

Total Pages in this Submission

35

TO THE ASSISTANT COMMISSIONER FOR PATENTS

Box Patent Application

Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

Microchip Device and Array

and invented by:

Cynthia Briscoe, Huinan Yu, Barbara Foley, Piotr Grodzinski, Jeremy Burton, Tony Chan and Rong-Fong Huang

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:

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Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 21 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☐ Cross References to Related Applications (if applicable)
 - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
 - d. ☐ Reference to Microfiche Appendix (if applicable)
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☒ Brief Description of the Drawings (if drawings filed)
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

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Application Elements (Continued)

3. ☒ Drawing(s) (when necessary as prescribed by 35 USC 113)
- a. ☒ Formal Number of Sheets 8
- b. ☐ Informal Number of Sheets _____
4. ☐ Oath or Declaration
- a. ☐ Newly executed (original or copy) ☐ Unexecuted
- b. ☐ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)
- c. ☐ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference (usable if Box 4b is checked)
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under
Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby
incorporated by reference therein.
6. ☐ Computer Program in Microfiche (Appendix)
7. ☐ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all must be included)
- a. ☐ Paper Copy
- b. ☐ Computer Readable Copy (identical to computer copy)
- c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(B) Statement (when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing
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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)

16. ☐ Additional Enclosures (please identify below):

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	32	- 20 =	12	x \$18.00	\$216.00
Indep. Claims	1	- 3 =	0	x \$78.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$760.00
OTHER FEE (specify purpose)					\$0.00
TOTAL FILING FEE					\$976.00

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Dated: December 17, 1999

cc:

CERTIFICATE OF MAILING

PATENT

Express Mail No. EL432914527US

Deposited: December 17, 1999

I hereby certify that the attached correspondence, identified below, is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

By: Rafael Rivera

In re the Application of: Briscoe et al.

For: Covalent Microchip Device and Array

Serial No. To be Assigned

- x Transmittal Letter
- x Patent Application (21 pages, including description, claims and abstract)
- x Drawings (8 sheets)
- x Return postcard

Attorney Docket No. 99,305

**APPLICATION FOR UNITED STATES LETTERS PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**
(Case No. 99,305)

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Title:

Microchip Device and Array

MICROCHIP DEVICE AND ARRAY

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to an apparatus for performing molecular reactions in which the reaction temperature must be changed at least once during the course of the reaction. Specifically, the invention relates to an apparatus in which the thermal conditions of a plurality of parallel molecular reactions can be independently controlled. More specifically, the invention relates to an apparatus on which the polymerase chain reaction can be performed. In particular, the invention provides a microchip array that is fabricated using ceramic multilayer technology and in which parallel, independently controlled molecular reactions, such as the polymerase chain reaction, can be performed.

2. Background of the Invention

The polymerase chain reaction (PCR) is a technique that permits amplification or detection of nucleic acid sequences. This technique has been applied to a wide variety of biological methods, including for example, DNA sequence analysis, probe generation, cloning of nucleic acid sequences, directed mutagenesis, detection of genetic mutations, diagnoses of viral infections, molecular "fingerprinting," and the monitoring of contaminating microorganisms.

The polymerase chain reaction comprises repeated rounds, or cycles, of target denaturation, primer annealing, and extension (Figure 1). This reaction process yields an exponential amplification of the desired target sequence and is most advantageously accomplished through the use of a thermally-stable polymerase. The length of time required to complete a particular PCR protocol is dependent upon the number of amplification cycles as well as the length of the denaturation, annealing, and extension steps. A typical PCR performed on a conventional thermal cycler can often take several hours.

The fidelity and efficiency of PCR amplification is affected by several factors. These factors include the concentration of various reaction components, particularly the polymerase, deoxynucleotide triphosphates, magnesium ions, target molecules, and amplimers (amplification primer pair), the length and temperature of the denaturation, annealing, and extension steps, the

number of cycles, and the specificity and length of the amplimers. Since the success of any given PCR amplification depends upon a number of variables, optimized reaction conditions are often empirically determined. However, such an optimization process is usually labor intensive, costly, and time consuming.

5 The development of microfabricated chemical reaction chambers and microfluidics systems have provided an attractive alternative to more conventional PCR techniques and equipment, resulting in improved control, speed, and efficiency. By utilizing PCR microchips fabricated on silicon or glass (Wilding et al., 1994, *Clin. Chem.* 40:1815-18; Shoffer et al., 1996, *Nucleic Acids Res.* 24:375-79; Cheng et al., 1996, *Nucleic Acids Res.* 24:380-85; Woodley et al., 10 1996, *Anal. Chem.* 68:4081-86; Northrup et al., 1998, *Anal. Chem.* 70:918-22; Ibrahim et al., 1998, *Anal. Chem.* 70:2013-17; U.S. Patent No. 5,498,392 (Wilding et al., 1996), U.S. Patent No. 5,587,128 (Wilding et al., 1996), U.S. Patent No. 5,589,136 (Northrup et al., 1996)), investigators have been able to reduce both the length of time and cost of performing amplification reactions. While conventional PCR is performed in volumes of between 10-100 15 μ L and require several hours to process, microchip PCR is performed in volumes of less than 5 μ L and can be completed in minutes. The decrease in reaction time for microchip PCR has been achieved as a result of the low thermal mass of silicon reaction chambers and the integration of thin-film heaters (Northrup et al., 1998, *Anal. Chem.* 70:918).

20 While silicon microchip arrays have been fabricated for the parallel analysis of multiple samples (Belgrader et al., 1998, *Clin. Chem.* 44:2191-94), such devices do not facilitate reaction condition optimization. In order to rapidly optimize amplification conditions for a particular target and amplimer pair, an investigator must be able to perform independently controlled, parallel amplifications on a single microchip array. Due to the inefficient well-to-well thermal isolation achievable in arrays constructed of silicon or glass and the complicated fabrication 25 methods required to prepare microchip arrays from such materials, present techniques have not permitted preparation of a cost-effective commercial microchip array for performing such optimization experiments. Thus, there remains a need in this art for a microchip array for performing independently controlled parallel reactions. Such a device would reduce the time and cost required to optimize amplification conditions using conventional PCR techniques and 30 equipment or currently available PCR microchip arrays.

SUMMARY OF THE INVENTION

This invention provides an apparatus upon which molecular reactions such as the polymerase chain reaction (PCR) can be performed. The apparatus of the invention is a microchip comprising one or a plurality of well structures, a cover or substance to seal the wells, a means for heating each well, a means for cooling each well, a means for monitoring the temperature of each well, and a means for delivering reagents into each well.

The microchip array of the invention is fabricated to have low thermal conductivity in order to minimize thermal crosstalk between adjacent chambers on the microchip, which permits independent thermal control of each microchip component. In preferred embodiments, the microchip of the present invention is fabricated using ceramic multilayer technology (as disclosed, for example, in co-owned and co-pending U.S. Serial Nos. 09/235,081 and 09/337,086, incorporated by reference herein). In additional preferred embodiments, the microchip array comprises air channels for thermally isolating microchip components. In still further preferred embodiments, the microchip array comprises thermal conducting material in thermal contact with each well on the microchip for removing heat therefrom and reducing thermal crosstalk between wells thereby. In some embodiments of the present invention, the biocompatibility of the ceramic material comprising the well structures may be enhanced by coating the microchip with a conformal compound such as parylene that reduces inhibition of the thermal molecular reactions within the ceramic wells.

The microchips of the invention comprise one or a plurality of wells. Preferably, the microchip possesses an array of wells in which parallel, independently controlled molecular reactions can be controlled by temperature cycling as required. For example, the microchip array of the present invention can be used to perform parallel, independently controlled PCR reactions, ligase chain reactions, or DNA ligations. Most preferably, the apparatus of the invention can be used to determine the optimal reaction conditions for the PCR amplification of a particular nucleic acid sequence. Alternatively, the invention can be used to perform multiple reactions under more than one set of amplification conditions.

In certain embodiments of the microchip arrays of the invention, the temperature of the wells is increased using an integrated heater. In preferred embodiments, the integrated heater is a resistive heater, and more preferably a thick film resistive heater plate. Alternatively, the wells can be heated through the use of metal lines integrated beneath the well or surrounding sides of

the wells, more preferably in a coil having one or more loops, in vertical or horizontal orientation. Parallel, variable heating of individual wells in a microchip array may be accomplished through the use of addressing schemes, preferably a column-and-row or individual electrical addressing scheme, in order to independently control the heat output of the resistive heaters in the vicinity of each well.

In certain embodiments of the microchip arrays of the invention, the temperature of the wells is decreased using an integrated cooler. In preferred embodiments, the integrated cooler is a metal via at the bottom of each well. In further preferred embodiments, the integrated cooler is a thermo-electric cooler attached to or integrated into the microchip beneath each well. Parallel, variable cooling of individual wells in a microchip array may be accomplished through the use of addressing schemes, preferably a column-and-row or individual electrical addressing scheme, in order to independently control heat dissipation using cooling elements in the vicinity of each well.

In preferred embodiments of the microchip arrays of the invention, the temperature of the well is monitored using an integrated resistive thermal detector or a thermocouple, advantageously molded into the microchip substrate in thermal contact and proximity to the well structures of the microchips of the invention. The resistive thermal detector can be fabricated from a commercially available paste that can be processed in a customized manner for any given design. Such thermocouples are commercially available in sizes of at least 250 microns, including the sheath. In certain alternative embodiments, the temperature of the wells is monitored using an integrated optical system, for example, an infrared-based system.

In certain embodiments of the microchip arrays of the invention, reagents can be deposited in appropriate regions or components, or can be delivered to said components from other components on the microchip. In preferred embodiments, reagents can be delivered to the wells of a microchip array using a microfluidic reagent distribution system. In preferred embodiments, said microfluidic distribution system is controlled by pressure, using pumping means, or by electro-osmotic pumping means, and fluid flow is controlled by valving, using a system of microfluidic channels and chambers to advantageously direct fluid flow on the microchip.

Compared with available prior art devices, the microchip arrays of the present invention will allow for more efficient and inexpensive performance of molecular reactions. For example,

the apparatus of the present invention can be used to perform PCR using reduced amounts of reagents in less time and with higher throughput than is possible using any commercially-available PCR machine. In addition, as a result of the fabrication techniques employed in the construction of the apparatus of the present invention, the microchip of the present invention is distinguished from prior art microchips in that an increased number of molecular reactions can be performed on a single microchip array. Finally, the addressable nature of the microchip array of the present invention allows for parallel optimization of molecular reaction conditions or the performance of simultaneous molecular reactions under variant reaction conditions.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic representation of a typical polymerase chain reaction amplification performed using the microchip arrays of the invention;

Figure 2 is a schematic representation of a cross-sectional view of a microchip array according to one embodiment of the invention;

Figure 3 is a schematic representation of the a cross sectional view of a microchip array according to one embodiment of the invention;

Figures 4A-4B are schematic representations of (A) a sixteen well microchip array and (B) a cross-sectional view of the embedded heating elements of a microchip array according to one embodiment of the invention;

Figure 5 is a schematic representation of a microchip array of the invention having column-and-row electrical addressing;

Figure 6 is a schematic representation of a microchip array with individual electrical addressing;

Figure 7 is a schematic representation of a cross-sectional view of a microchip well structure and integrated heating and cooling elements.

Figures 8A-8C illustrate the thermal cycling capability of the microchip device of the invention during a 25-cycle experiment (Figure 8A), over the course of 2 cycles in a 25-cycle experiment (Figure 8B), and over the course of 2 cycles in a 25-cycle experiment in which the microchip device was clamped to a commercially available thermal cycler (Figure 8C). In all experiments illustrated, a cycle consisted of a "denaturation" step of 45 sec. at 94°C and an "annealing" step of 60 sec. at 72°C.

Figure 9 illustrates the results obtained for the PCR amplification of *bla* using the microchip device of the present invention, the left-hand lane contains fragment size standards.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The preferred embodiment of the present invention and its advantages over previously investigated PCR microchip devices are best understood by referring to Figures 2-7 and Examples 1 and 2.

Figure 2 is a schematic representation of a cross-sectional view of the PCR microchip 1 of the present invention. The microchip 1 is built on a layer of thermal insulating material 2, that is most preferably made of glass, silicon, plastic, or ceramic. In a preferred embodiment, this layer is made of ceramic. As ceramic materials are intrinsically good thermal insulators, a thermal insulating layer made of ceramic provides good well-to-well thermal insulation that is a requirement for performing parallel, independent PCR amplifications on a single microchip. As the thermal conductivity of silicon is about eleven times greater than that of ceramics, the multilayer ceramic microarray of the present invention has an advantage over prior art devices constructed of silicon in that an increased number of well structures for performing molecular reactions may be placed onto an array of significantly reduced size. In addition, the multilayer ceramic microarrays of the present invention have an advantage over prior art devices constructed of silicon in that electrical cross-talk is lower in the ceramic microarrays.

Furthermore, the ceramic microarrays of the present invention are more biocompatible than the silicon microarrays of prior art devices.

In a preferred embodiment, the ceramic layer of the microchip of the present invention is fabricated using multilayer ceramics technology as disclosed in co-owned and co-pending U.S. Patent Applications, Serial Nos. 09/235,081 and 09/337,086 (Burdon *et al.*), the disclosures of which are explicitly incorporated by reference herein. The invention disclosed therein relates to a method for the fabrication of a multilayered microfluidic device. A plurality of green-sheet layers, consisting of ceramic, is textured in a first predetermined pattern defining fluid passageways. A thick-film paste is applied to the green-sheet layers in a second predetermined pattern defining fluid-interacting components. The green-sheet layers are then sintered together at a predetermined temperature for a predetermined amount of time to form a substantially monolithic structure, having fluid passageways and fluid-interacting components defined therein.

By allowing each green-sheet layer to be processed individually before being sintered together, complicated structures can be built into the multilayered microfluidic devices of the invention disclosed by Burdon et al. For example, the fluid passageway in the device can be defined by structures, such as vias and channels, which are formed into several green-sheet layers before the layers are sintered together. In this manner, fabrication out of a plurality of layers allows the fluid passageway to have a complicated three-dimensional structure that would be difficult to achieve through the use of more conventional materials.

The technology disclosed by Burdon et al. also permits incorporation of a wide variety of functional components, such as heating elements, cooling elements, fluid sensors, and fluid motion sensors that would be useful and/or necessary for the fabrication of a microchip for performing molecular reactions. To provide these integral components, thick-film pastes can be silk-screened onto individual green-sheet layers and then co-fired with and sintered to the green-sheet layers to become. For example, the thick-films can include conductive materials, such as metals, to provide thermoelectrically conductive pathways in the device.

The PCR microchip **1** of the present invention contains one or more well structures **3**, in which PCR amplifications can be performed. As used herein, “wells” and “well structures” refer to a hole or open area in the substrate of the microchip that is used to contain, mix, or react reagents comprising the molecular reactions performed on the microchip. Typically, wells have

a depth of about 2 mm and a diameter of about 1 mm, with a volume of 1-5 μ L, more preferably about 2 μ L, and may be configured as, for example, cylinders, rectangles, or squares.

In some embodiments well structures are formed from a thermal conducting material such as undoped silicon, metals, or modified plastics. In preferred embodiments, the well structures are formed from metals. In more preferred embodiments the metal is silver or silver palladium (containing up to 30% palladium). In other preferred embodiments, the well structures are formed from copper, Ni-Molybdenum, platinum, or gold. Typical formulations of such materials for the fabrication of the well structures of the apparatus of the present invention can be obtained from thick film manufacturers such as DuPont (Research Triangle Park, NC) or Hereaus (West Conshohocken, PA).

Well structures comprised of a thermal conducting material are separated on the microchip by channels 4 comprising thermal insulating material such as glass, silicon, plastic, ceramic, or air contained in air channel components of the microchip. As used herein, the term "channel" and "microchannel" refers to an open region within the substrate of the microchip having a length greater than its diameter. Typical air channels have diameters ranging from 100 to 500 microns. As used herein, channels and microchannels can contain fluids or gasses, and can be used to move fluids or gasses between components on the microchip.

In a preferred embodiment, the thermal insulating material 4 used to separate the well structures comprises air contained in said air channels (Fig. 3). In one preferred embodiment, the air channels have a width of at least 75 microns. Since air has a poor thermal conductivity, air channels of this dimension would be useful in reducing the thermal cross-talk between the plurality of well structures of the microchip array of the present invention. Furthermore, the multilayer ceramic microarrays of the present invention have an advantage over prior art devices constructed of silicon in that the fabrication of air channels produces a channel of more uniform dimensions.

Where air channels are used for thermal insulation in the multilayer microfluidics devices of the present invention, the channels can be, for example, cylinders, rectangles, or squares, or any other convenient or useful cross-sectional shape, and the channels are limited by the requirement that at least one vertex is attached to the green-sheet layer from which the channel has been formed. As a result of this limitation, air structures in the microchip array of the

present invention are not fabricated to completely surround any well structure without permitting at least one vertex between the well structure and the green-sheet layer to be maintained.

The temperature of the well structures can be increased using an integrated heater. In preferred embodiments, the integrated heater is a resistive heater, and more preferably a thick film resistive heater plate. In alternative and preferred embodiments, the wells are heated through the use of metal lines integrated beneath the well or surrounding sides of the wells, preferably in a coil having one or more loops in vertical or horizontal orientation. In a preferred embodiment, the well structures are heated by metal lines 5 that are integrated beneath the wells or on the sides of wells.

The temperature of the well structures can be decreased using an integrated cooler. In preferred embodiments, the integrated cooler is a metal via at the bottom of each well. Optionally, the metal via is in thermal contact with a metal plate, an array of metal discs or a thermo-electric cooler, each of which functions as a heat sink or an active cooling means. Commercially-available thermo-electric coolers can also be incorporated into the inventive apparatus, because they can be obtained in a wide range of dimensions, including components of a size required for the fabrication of the microarrays of the present invention. In embodiments comprising metal heat sinks encompassing a metal plate or an array of metal discs, the plate or discs are composed of iron, aluminum, or other suitable metal.

As used herein, the term "via" refers to a hole formed in the green-sheet layer that when sintered together with other green-sheet layers comprises the multilayered microfluidic microchip device for performing molecular reactions of the present invention. Typical vias have diameters ranging from 100 to 500 microns. Vias may also be filled with other materials, such as metallic pastes containing metal particles, such as silver, platinum, gold, copper, tungsten, nickel, tin, or alloys thereof. Preferably the metallic paste is silver.

An integrated temperature sensor or thermosensor monitors the temperature of each of the well structures on the microchips of the invention. In preferred embodiments, the integrated thermosensor is a thermoelectric, optical or electrochemical sensor as illustrated as component 6 in Figure 4B. Alternatively, the temperature of the well is monitored using an integrated resistive thermal detector or a thermocouple, advantageously molded into the microchip substrate in thermal contact and proximity to the well structures of the microchips of the invention.

In a preferred embodiment, a cover 7 seals the PCR microchip 1 of the present invention. In some embodiments, certain components of the heating, cooling, or temperature monitoring systems are integrated into the cover. In still other embodiments of the present invention, a separate heating system to prevent condensation of the reaction mixture onto the cover is incorporated into the cover itself. Alternatively, a covering of mineral oil in individual wells can be used in place of the cover of the preferred embodiment.

A preferred embodiment of the microchips of the present invention is a PCR microchip array comprising a plurality of well structures in which parallel, independent amplification reactions can be performed. In certain and preferred embodiments, heating of the microchip array is accomplished through column-and-row electrical addressing of individual well structures. In alternative preferred embodiments, the well structures are each individually addressed. Figure 5 illustrates a schematic representation of a microchip array with column-and-row electrical addressing. Figure 6 illustrates a schematic representation of a microchip array with individual cell electrical addressing. In contrast to column-and-row addressing, an individual addressing configuration allows for the independent heating of each individual well structure.

To fabricate glass or silicon microchips for use in parallel, independently controlled molecular reactions a complex arrangement of heating elements would be required. However, in a preferred embodiment, multilayer ceramics technology permits electrical connections to individual well structures to be distributed three-dimensionally in the microchip.

Figure 7 is a schematic representation of a cross-sectional view of one embodiment of the well structure and integrated heating and cooling elements associated therewith of the microchip array of the present invention. In this embodiment of the present invention, the heating elements are wrapped around the perimeter of the well and form a spiral from top to bottom (as further illustrated in Figure 4B).

The well structures of the microarray of the present invention can have volumes ranging from 1 to 25 μL , and may be configured as, for example, cylinders, rectangles, or squares, or any other convenient or useful cross-sectional shape. In one embodiment of the present invention, the well structures have a volume of about 2 μL and are configured as cylinders. Suitable well structures may have a number of different dimensions that would permit reactions of between 1 and 25 μL to be performed therein. In preferred embodiments of the microarray of the present

invention, the well structures have depths of between 1 and 10 mm and diameters of between 0.5 and 5 mm. In one embodiment, the well structures have a depth of 2 mm and a diameter of 1.2 mm. In an alternative embodiment, well structures have a depth of 2.5 mm and a diameter of 1 mm (Fig. 7). The flow of heat will determine the most favorable dimensions for the well structures, and the dimensions will vary with the materials used for the fabrication of the integral heating and cooling components.

The integrated heaters of the well structures can be fabricated from metallic pastes containing metal particles, such as silver, platinum, gold, copper, tungsten, nickel, tin, or alloys thereof. Preferably the integrated heaters are fabricated from a metallic paste that is silver. In preferred embodiments, the integrated heaters comprise a lead that is about 30 wide mil, connected to a resistive heater that is about 5 mil wide. This arrangement is shown in Figure 4B.

Also provided are resistive thermal devices, for monitoring the thermal energy and temperature produced by the resistive heaters. The RTD, that senses the heat produced by the heater, has a lead that is 10-20 mil wide, a body of the RTD is 5 mil wide and is about 8-15 microns thick. This arrangement is also shown in Figure 4B

In the preferred embodiment of the present invention, the supporting substrate has a surface area of between 1 and 100 cm² containing between 1 and 500 well structures having the shape and dimensions as disclosed herein. In the most preferred embodiments, the well structures are arranged on the substrate so as to be separated by a distance of between 0.1 to 10 mm. In more preferred embodiments, the well structures are separated by channels of insulated material having the shape and dimensions as disclosed herein and the channels and well structures are separated by a distance of between 0.1 and 10 mm. Most preferably, the well structures are regularly spaced on the solid substrate with a uniform spacing there between.

The Examples, that follow, are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Thermal Cycling Capability of Ceramic Microchip Device

The thermal cycling capability of the microchip device of the invention was examined as follows. A ceramic microchip device was constructed as described herein. The temperature of the device was regulated using a controller and computer as described below or by clamping the device onto a commercially available thermal cycler (MJ Research, Inc., Waltham, MA). The temperature of the device was monitored using a resistive temperature device paste (RTD; DuPont part number 5092D) having a coefficient of 3000 ± 200 ppm/C. The microchip device was fabricated by printing the RTD paste onto the device twice in order to achieve a lower resistance value. The typical resistance of the printed RTD element on the microchip device was 300 ohm.

A multi-loop controller (MOD30ML) from Asea Brown Boveri Ltd. (ABB; Norwalk, CT; <http://www.abb.com/global/usabb/usabb045.nsf?OpenDatabase&db=/Global/USABB/u>) was used to perform the temperature control process. Temperature and time control was performed using a proportional integral differentiate (PID) algorithm available within the ABB controller. The software for time step and temperature setpoint control was written using "Application Builder" software purchased from ABB. This software allowed the time and temperature setpoint to be specified, modified and controlled using a personal computer. The computer graphical user interface that allowed setup and modification of PCR thermal procedures in real time (allowing flexible automation of the entire reaction) was Fix32, purchased from Intellution, Inc. This software is a general purpose automation control software that allows users to customize the graphical display. Data acquisition was done using the computer serial port, and thus needed no additional computer hardware components.

The thermal cycling capability of the microchip device was analyzed over the course of a 25-cycle experiment in which each cycle consisted of a "denaturation" step of 45 sec. at 94°C and an "annealing/extension" step of 60 sec. at 72°C . For each experiment, the well structure of the microchip device contained $1\ \mu\text{L}$ of PCR mix (see Example 2) and $0.5\ \mu\text{L}$ of chill-out liquid wax (MJ Research). Figures 8A-8C illustrate the thermal cycling capability of the microchip device of the invention during a 25-cycle experiment (Figure 8A), over the course of 2 cycles in a 25-cycle experiment (Figure 8B), and over the course of 2 cycles in a 25-cycle experiment in which the microchip device was attached to a commercially available thermal cycler (Figure 8C).

The microchip device was attached to the thermal cyclor as follows. A sufficient amount of mineral oil was placed on the temperature block of a thermal cyclor (MJ Research) to create a thermal connection between the microchip device and the temperature block. Mineral oil was first placed on the flat temperature block, and the array containing all required samples and reagents was then placed on top of the mineral oil layer. The lid of the thermal cyclor was then closed. The thermal cyclor controlled time and temperature variations on the microchip array; the thermal detector of the microchip array was engaged to monitor temperature changes and rates of temperature changes on the array. The temperature data was collected from the array as described above, and is shown in Figure 8C.

The results of the performance of a PCR reaction as describe above are shown over 25 cycles (Figure 8A) and 2 cycles (Figure 8B). As shown in these Figures, the temperature set by the controller and computer compared favorably with that measured by the RTD, thus indicating that the microchip device of the present invention could be applied in for PCR amplification of nucleic acids. These results illustrate the rapid rates of temperature change that can be effected using the microchip arrays of the invention. As a consequence, the amount of time the reaction is maintained at the appropriate denaturation and annealing/extension temperatures is maximized, thus minimizing overall cycle times and reaction times.

In contrast, the data in Figure 8C demonstrated that rates of temperature change are much slower using the thermal cyclor than the rates obtained using the microchip itself. Due to this intrinsic inefficiency, the thermal cyclor requires more cycle time and overall reaction time to achieve the same degree of fragment amplification.

EXAMPLE 2

Polymerase Chain Reaction Amplification of *bla* on Ceramic Microchip Device

The application of the microchip device of the invention as a device for performing the polymerase chain reaction was examined as follows. A ceramic microchip device was constructed as described herein, and thermal cycling was controlled as described in Example 1.

A two-step PCR protocol was performed to amplify a 627bp fragment of the plasmid marker β -lactamase (*bla*) encoding the gene responsible for ampicillin resistance (AmpR) carried by the *E. coli* K12 strain, DH5 α on plasmid pBluescript KS+ using a kit obtained from Perkin

Elmer (Norwalk, CT). PCR was performed for a total of twenty-five cycles, where each cycle consisted of a "denaturation" step of 45 sec. at 94°C and an "annealing" step of 60 sec. at 72°C (wherein primer annealing and extension were performed at the same temperature). A 50 µL PCR reaction mixture containing *bla*-specific primers (BLA-fl + BLA-r1, contained in the Perkin Elmer kit) was prepared according to manufacturer's instructions, and 1µL of this mixture was introduced into one of the wells of a ceramic microarray of the invention. The reaction mix in the microchip was covered with 0.5 µL of chill-out liquid and then was amplified as described in Example 1. The remaining portion of the mixture was placed in a standard PCR tube and PCR performed in a conventional thermal cycler (MJ Research).

After the amplification reaction was completed, the reaction products from the microarray and the thermal cycler were analyzed by 4-20% polyacrylamide gel/Tris-borate EDTA gradient gel electrophoresis and visualized with an intercalating dye (SyBr-Green) using a Molecular Dynamics FluorImager set at 488 nm and appropriate calibration filters. Figure 9 illustrates the results obtained for the PCR amplification of *bla* using the microchip device of the present invention (Fig. 9, lane 4) and the conventional thermal cycler (Fig. 9, lanes 2 and 3; lane 2 contains 10µL of the reaction mixture and lane 3 contains 1µL of the reaction mixture). The expected *bla* PCR product (627 bp) was obtained using the microchip device, thus indicating that the microchip device of the present invention can be used for PCR amplification of nucleic acids.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WHAT WE CLAIM IS:

1. An apparatus for performing parallel, independently controlled molecular reactions, comprising:

- 5 (a) a supporting substrate comprising a ceramic layer,
- (b) a plurality of separate well structures comprising a thermal conducting layer and wherein the well structures are separated by a thermal insulating layer,
- (c) a means for heating each well structure in thermal contact with the thermal conducting layer comprising each well,
- 10 (d) a means for cooling each well structure in thermal contact with the thermal conducting layer comprising each well, and
- (e) a means for monitoring the temperature of the contents of each well structure in thermal contact with the thermal conducting layer comprising each well.

15 2. The apparatus of Claim 1, wherein the molecular reaction is polymerase chain reaction.

3. The apparatus of Claim 1, wherein the supporting substrate is made of a thermal insulating material.

20 4. The apparatus of Claim 3, wherein the thermal insulating material is glass, silicon, plastic, or ceramic.

25 5. The apparatus of Claim 1, wherein the thermal insulating material is fabricated using ceramic multilayer technology.

6. The apparatus of Claim 1, wherein the thermal conducting layer of the well structures contain a resistive heater.

30 7. The apparatus of Claim 6, wherein the well structures are made of a thermal conducting material and are separated by a thermal insulating material.

8. The apparatus of Claim 7, wherein the thermal insulating material is glass, silicon, plastic, or ceramic.

9. The apparatus of Claim 7, wherein the thermal insulating material is fabricated using ceramic multilayer technology.

10. The apparatus of Claim 6, wherein the thermal conducting material is undoped silicon, modified plastics, silver, silver palladium, copper, nickel-molybdenum, platinum, or gold and the thermal insulating material is glass, silicon, plastic, ceramic, or air contained in an air channel positioned proximal to the well structure.

11. The apparatus of Claim 1, wherein the well structures are coated with a compound that enhances biocompatibility between the components of the molecular reaction and the thermal insulating or conducting material comprising the well structures.

12. The apparatus of Claim 11, wherein the compound coating is parylene.

13. The apparatus of Claim 1, wherein the means for heating each well structure is an integrated heating system.

14. The apparatus of Claim 13, wherein the integrated heating system is a thin film resistive heater.

15. The apparatus of Claim 13, wherein the integrated heating system is a metal wire resistive heater.

16. The apparatus of Claim 15, wherein the metal wire resistive heater is integrated into the thermal insulating material comprising the supporting substrate.

17. The apparatus of Claim 13, wherein the integrated heating system utilizes column-and-row electrical addressing.

18. The apparatus of Claim 13, wherein the integrated heating system utilizes individual electrical addressing.

5 19. The apparatus of Claim 1, wherein the means for cooling each well structure is a passive cooling system.

20. The apparatus of Claim 1, wherein the means for cooling each well structure is an active cooling system.

10 21. The apparatus of Claim 20, wherein the active cooling system is an integrated cooling system.

15 22. The apparatus of Claim 21, wherein the integrated cooling system comprises a metal plate, and array of metal discs, or a thermo-electric cooler, wherein the integrated cooling system is in thermal contact with each of the well structures.

20 23. The apparatus of Claim 1, wherein the means for monitoring the temperature of the molecular reactions in each well structure is an integrated optical or electrochemical sensor system.

24. The apparatus of Claim 1, further comprising a means for delivering reagents into each well structure.

25 25. The apparatus of Claim 23, wherein the means for delivering reagents into each well structure is a microfluidic reagent distribution system.

26. The apparatus of Claim 1, further comprising sealed well structures.

30 27. The apparatus of Claim 26, wherein the well structures are sealed using a layer of mineral oil.

28. The apparatus of Claim 26, wherein the well structures are sealed using a cover.

29. The apparatus of Claim 28, wherein the cover further comprises a means for
5 heating the well structures.

30. The apparatus of Claim 28, wherein the means for heating the well structures is an
integrated heating system.

10 31. The apparatus of Claim 4, wherein the thermal insulating material is plastic and
the means for monitoring the temperature of the contents of each well structure is an integrated
resistive thermal detector which can be molded into the plastic.

15 32. The apparatus of Claim 4, wherein the thermal insulating material is plastic and
the means for monitoring the temperature of the contents of each well structure is an
thermocouple which can be molded into the plastic.

[illegible]

5

Fig. 1

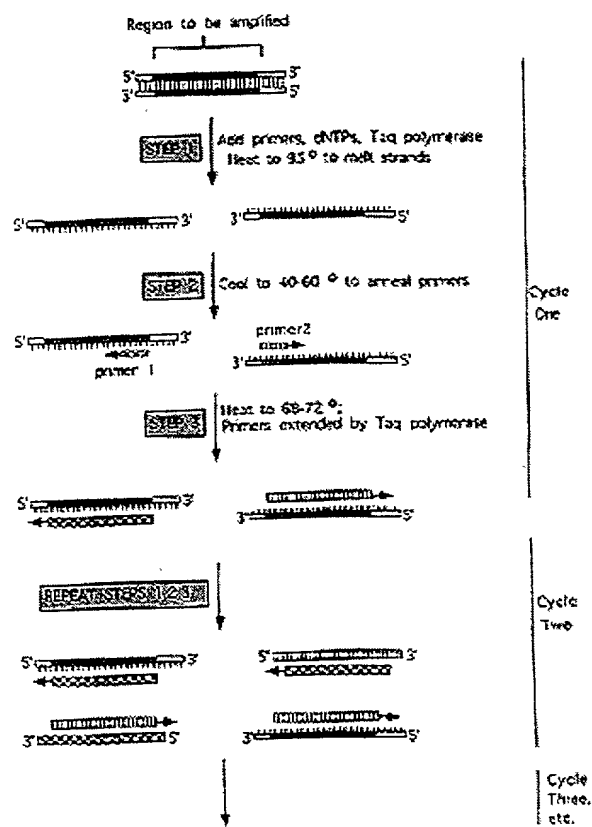
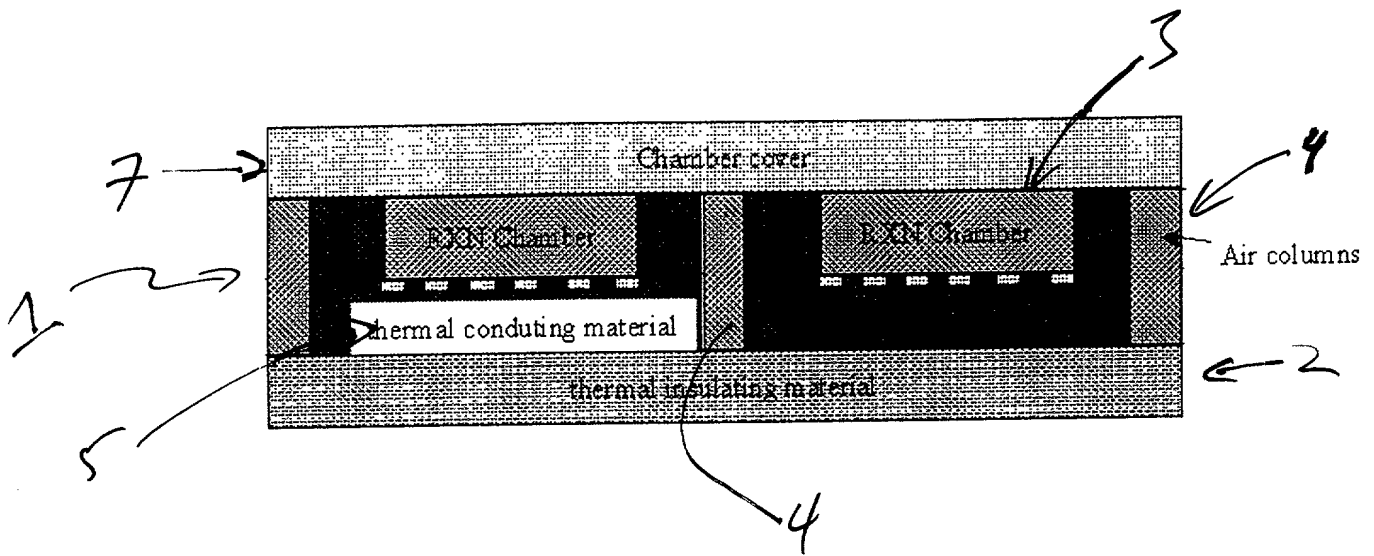


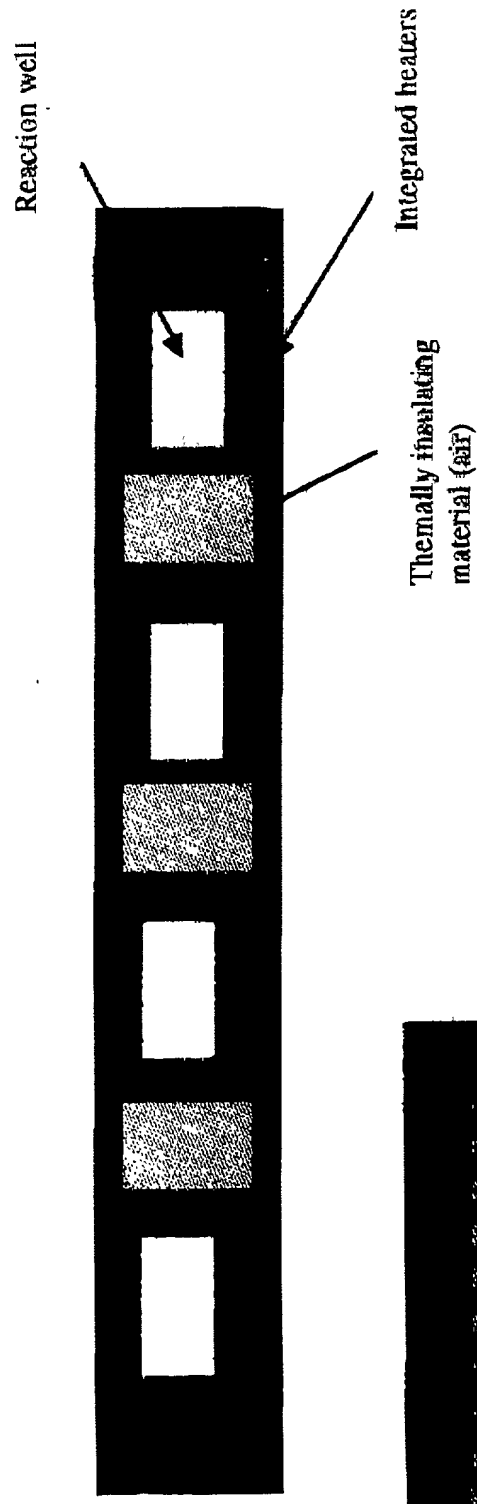
Fig. 2



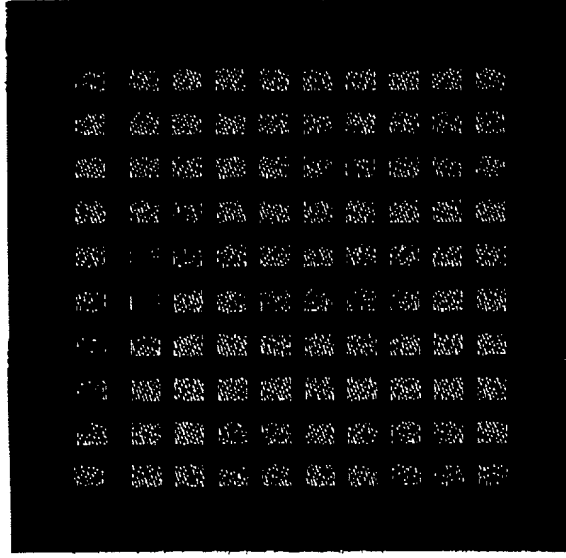
09466329 121799

Fig. 3

Microchip PCR Array



- Integrated & independent heaters
- Multiple sample parallel testing (high throughput)

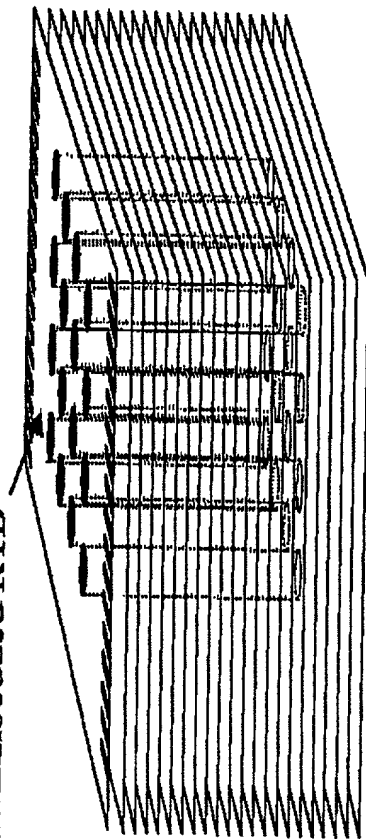


Example of column - row addressing

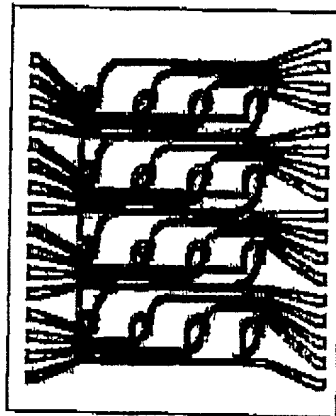
Ceramic micro-PCR Heater Array-Final Design

4 X 4 Array of Reaction Wells
with Embedded Heaters and
an Embedded RTD

Fig. 4A

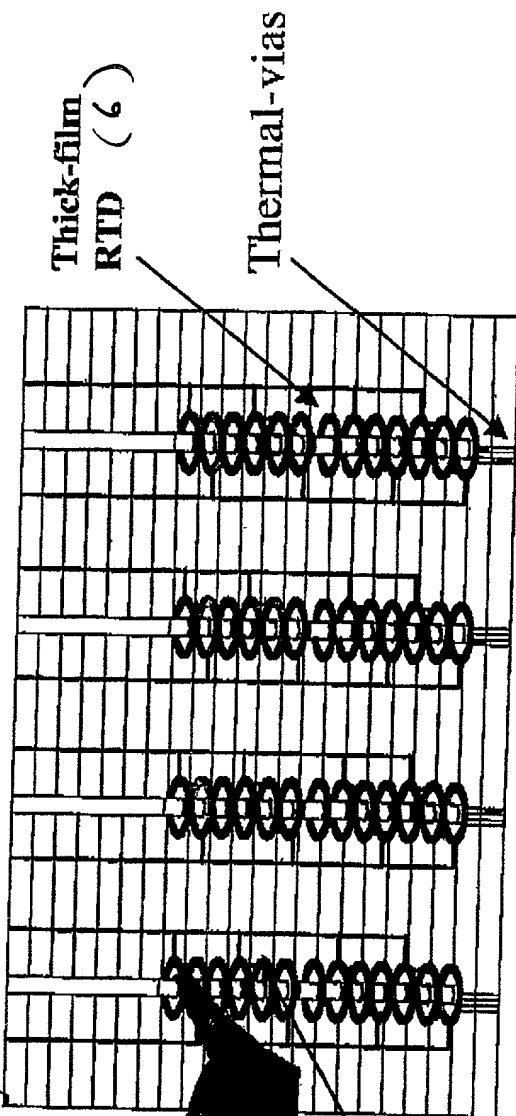


Device Anatomy



Embedded Heaters-
4 Sets of Heaters in Parallel
3 Heaters in Each Set in Series

Fig. 4B



Thick-film
RTD (6)

Thermal-vias

Fig. 5

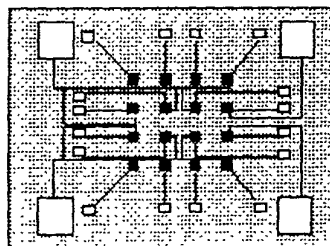
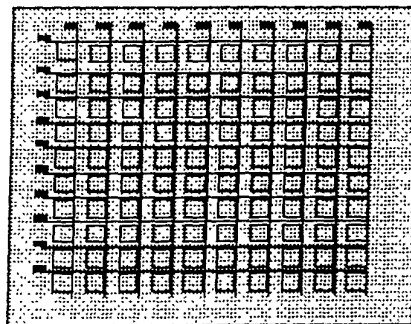
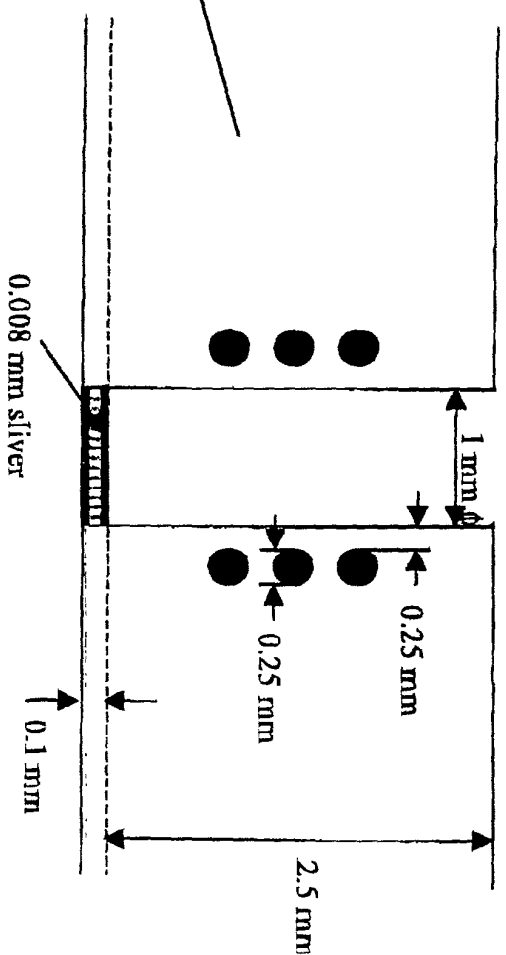
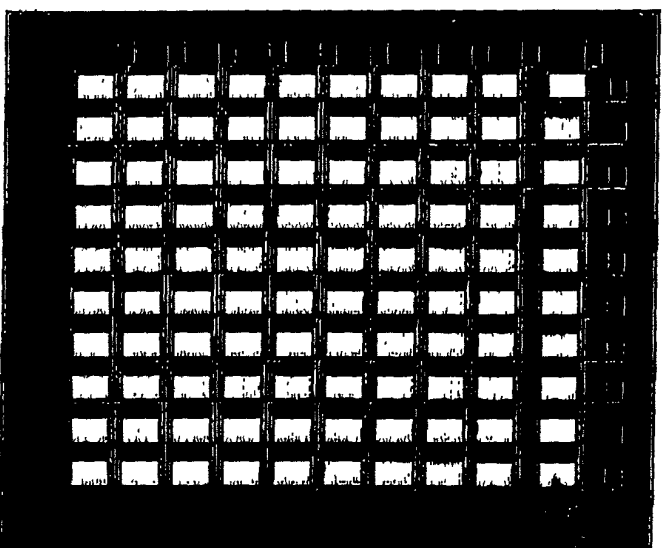


Fig. 6

Schematic of Array with Integrated Heaters with Active Cooling



Fig. 7



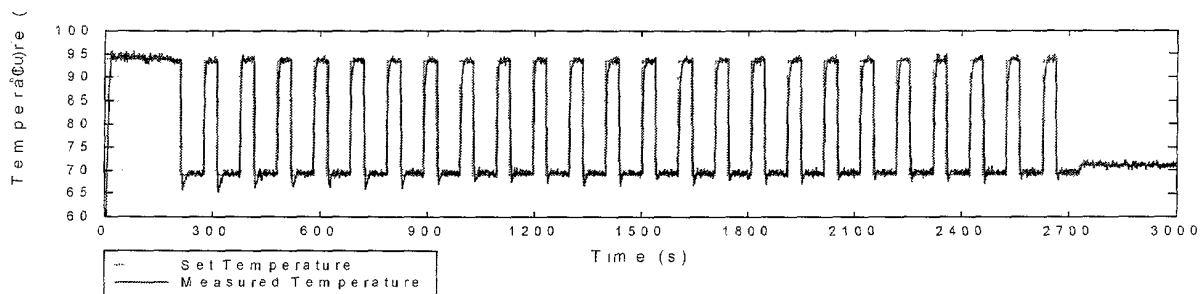


Fig. 8A

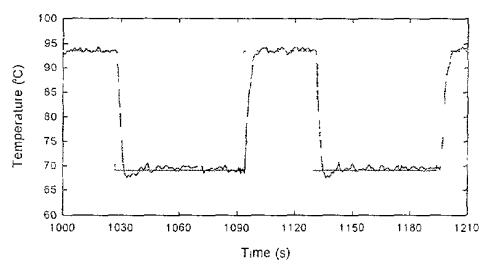


Fig. 8B

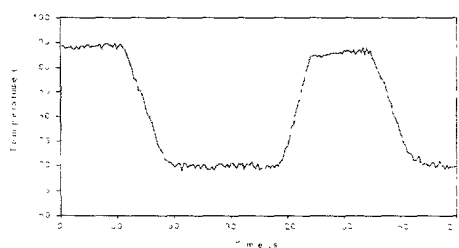


Fig. 8C

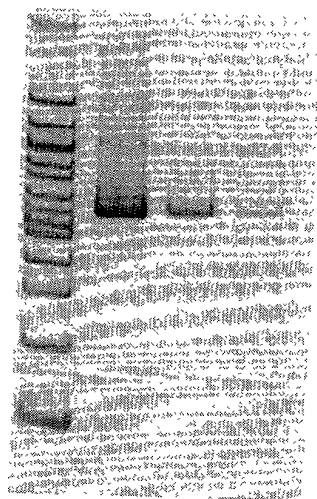


Fig. 9